410 Rec'd PCT/PTO 28 AUG 2000

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
KINOSHITA3

U.S. APPLICATION NO (If known, see 37 CFR 1.5)
09/623138

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY CLAIMED
PCT/JP99/00833	24 February ~~2000~~ 1999	26 February 1998

TITLE OF INVENTION
LANGERHANS CELLS MIGRATION INHIBITORS

APPLICANT(S) FOR DO/EO/US
S. KINOSHITA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [xx] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [xx] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [xx] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [xx] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [xx] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [xx] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [xx] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [xx] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [xx] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [xx] A FIRST preliminary amendment.
    [xx] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [xx] Other items or information:
    [xx] Courtesy copy of the first page of the International Publication (WO 99/43330).
    [xx] Courtesy copy of the International Preliminary Examination Report. There were no annexes.
    [xx] Formal drawings, 5 sheets, Figures 1-5.
    [xx] Courtesy Copy of the International Search Report.

U.S. APPLICATION NO. (If known,see 37 CFR 1 5)	International Application No	Attorney's Docket No
09/623138	PCT/JP99/00833	KINOSHITA3

17. [XX] The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) –(5):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$970.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$840.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$760.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$670.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				$	840.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
Claims as Originally Presented	Number Filed	Number Extra	Rate		
Total Claims	10 - 20	0	X $18.00	$	0
Independent Claims	1 - 3	0	X $78.00	$	0
Multiple Dependent Claims (if applicable)			+$260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$	840.00
Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate		
Total Claims	10 - 20	0	X $18.00	$	0
Independent Claims	1 - 3	0	X $78.00	$	0
TOTAL OF ABOVE CALCULATIONS =				$	840.00
Reduction of ½ for filing by small entity, if applicable. A small entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)				$	
SUBTOTAL =				$	840.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	840.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$	840.00
				Amount to be: refunded	$
				charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [XX] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of $840.00, is attached.

c. [ ] Please charge my Deposit Account No. **02-4035** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. **[XX]** The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Date of this submission: **August 28, 2000**

S.N. 19,963 F09

SIGNATURE

Roger L. Browdy

NAME

25,618

REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: S. KINOSHITA	)	Art Unit:
IA No.: PCT/JP99/00833	)	Washington, D.C.
IA Filed: 24 February 1998	)	
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	August 28, 2000
For: LANGERHANS CELL …	)	Docket No.: KINOSHITA3

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

--CROSS REFERENCE TO RELATED APPLICATION

The present application is the national stage under 35 U.S.C. 371 of PCT/JP99/00833, filed 24 February 1999. --

IN THE CLAIMS

Claim 5, lines 1-2, delete "any one of claims 1 to 4", and insert therefor --claim 1--.

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: S. KINOSHITA	)	Art Unit:
IA No.: PCT/JP99/00833	)	Washington, D.C.
IA Filed: 24 February 1998	)	
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	August 28, 2000
For: LANGERHANS CELL …	)	Docket No.: KINOSHITA3

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please add the following claims:

--11. A method for prevention and/or treatment of skin or ocular inflammation in a mammal comprising administering an effective amount of a langerhans cell migration inhibitor comprising active vitamin D as an active ingredient to the mammal.

--12. The method of Claim 11 wherein the active vitamin D is vitamin D_3 or a derivative or analog of vitamin D_3.

--13. The method of Claim 12 wherein the active vitamin D is vitamin D_3.

--14. The method of Claim 13 wherein the active vitamin D_3 is calcitriol or 22-oxacalcitriol.

--15. The method of Claim 14 wherein the inhibitor is in the form of an ophthalmic solution.

--16. The method of Claim 15 wherein the inhibitor is used for the prevention and/or treatment of ocular inflammation.

--17. The method of Claim 16 wherein the ocular inflammation is keratoconjunctivitis.

--18. The method of Claim 15 wherein the inhibitor is used for the prevention of ocular inflammation.

--19. The method of Claim 18 wherein the ocular inflammation is phlyctenular keratitis or corneal infiltration.

--20. The method of Claim 15 wherein the inhibitor prevents and/or treats an ocular inflammation by inhibiting the production of interleukin-1 in corneal epithelial cells.--

REMARKS

Claims 11-20 presently appear in this case.

The above amendments to the claims are being made in order to place the application in better condition for examination.

Favorable consideration is earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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SPECIFICATION

LANGERHANS CELL MIGRATION INHIBITORS

FIELD OF THE INVENTION

The present invention relates to Langerhans cell migration inhibitors, and more specifically, Langerhans cell migration inhibitors comprising active vitamin D as an active ingredient.

PRIOR ART

Langerhans cells take up a foreign antigen into the skin, move to lymphatic ducts, where they become veiled cells to intracellularly partially degrade the antigen, and then move to adjacent lymphatic organs to present the partially degraded antigen to T cells whereby the T cells are differentiated into contact dermatitis-inducing cells. Langerhans cells are also known to migrate from the corneal limbus into the central cornea, where they produce various cytokines to induce immunoreactive inflammation.

Since Langerhans cells are deeply involved in the development stage of immunoreactive inflammation of the skin and cornea as described above, continued limitation of the number of these cells at the entry site of foreign antigens seems to prevent the inflammation at the surrounding sites. Especially, it is important to prevent corneal inflammation, which affects corneal transparency and thus lowers the vision of the patient.

At present, potent drugs such as steroids and cyclospolin A are used to inhibit immunoreaction in the skin or cornea, but these drugs are inappropriate for use before

inflammation occurs because of their various side effects. Therefore, there is a demand for development of a drug with less side effects that can be safely used before inflammation occurs.

USP. No. 4,610,478 discloses a composition for topical treatment of dermatitis, comprising active vitamin D, 1-α-hydroxylcholecalciferol or 1-α,25-dihydroxylcholecalciferol.

JP No. 503922/93A (WO91/05537) discloses a method for promoting the cure of wounds, comprising administering a vitamin D compound to the patient.

WO96/29079 discloses an opthalmic composition for treating inflammation in the anterior part of the eyeball, comprising an active vitamin D as an active ingredient.

These publications disclose the antiinflammatory agents with less side effects comprising an active vitamin D instead of steroids and therapies using them, but all of them are directed to a therapy of inflammation already developed and none of them suggest inhibition of migration of Langerhans cells that are deeply involved in the development stage of immunoreactive inflammation.

An object of the present invention is to provide a pharmaceutical composition with less side effects that can prevent immunoreactive inflammation of the skin or cornea and also can cure the inflammation after it occurs.

SUMMARY OF THE INVENTION

The present invention provides a Langerhans cell migration inhibitor comprising an active vitamin D as an

active ingredient. This active vitamin D is preferably active vitamin D_3 or a derivative or analog of vitamin D_3, more preferably active vitamin D_3, and most preferably calcitriol or 22-oxacalcitriol.

The Langerhans cell migration inhibitor of the present invention can be preferably in the form of an ophthalmic solution, and can be used for the prevention or treatment of ocular inflammation, particularly keratoconjunctivitis as well as the prevention of phlyctenular keratitis or corneal infiltration.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the score of neovascularization in murine corneas in Test example 1. The ordinate indicates the score and the abscissa indicates the number of days after stitching.

FIG. 2 is a graph showing the effect of calcitriol ($1\alpha,25(OH)_2D_3$) on the proliferation of human corneal epithelial cells in Test example 2.

FIG. 3 is a graph showing the effect of calcitriol on IL-1α production in Test example 2.

FIG. 4 is a graph showing the effect of calcitriol on IL-1β production in Test example 2.

FIG. 5 is a graph showing the effect of calcitriol on IL-8 production in Test example 2.

DETAILED DESCRIPTION OF THE INVENTION

Examples of inflammation to which Langerhans cell migration inhibitors of the present invention can be applied include inflammations of the surface layer of the cornea or

conjunctiva such as allergic keratoconjunctivitis, spring catarrh and diffuse keratoconjunctivitis, and inflammations reaching the corneal stroma such as corneal degeneration, corneal infiltration and phlyctenular keratitis. Especially, they are preferably applied to allergic keratoconjunctivitis.

Examples of active vitamin D which is an active ingredient of the composition of the present invention include cholecalciferol derivatives, cholecalciferol analogs, ergocalciferol derivatives, ergocalciferol analogs and hydrophilic active vitamin D analogs having a hydrophilic group at the side chain. These active vitamins D may be natural or synthesized. Specific examples of the active vitamin D include calcitriol (1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$)), 1α,24-dihydroxyvitamin D_3, alphacalcidol (1α-hydroxyvitamin D_3), calcifedol (25-hydroxyvitamin D_3), 1α,25,26-trihydroxyvitamin D_3, 1β,25-dihydroxyvitamin D_3, 24-homo-1α,25-dihydroxyvitamin D_3, 26-homo-1α,25-dihydroxyvitamin D_3, OCT (22-oxacalcitriol), and calcipotriol. Preferred active vitamins D are vitamin D_3, vitamin D_3 derivatives and vitamin D_3 analogs, specifically, calcitriol and 22-oxacalcitriol.

The Langerhans cell migration inhibitor of the present invention is generally used in the form of a pharmaceutical composition comprising active vitamin D in combination with a pharmaceutical carrier and formulated as a solution or ointment for topical administration. The compositions are preferably formulated as an ophthalmic liniment, especially an ophthalmic solution. Examples of

carriers suitable for these formulations include, but not limited to, water, ethanol, ethylene glycol, propylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone, gum arabic, calcium phosphate, alginate, gum tragacanth, gelatin, methylcellulose, talc, magnesium stearate, hyaluronic acid, chondroitin sulfate, collagen, fat and mineral oils. In addition to these carriers, the pharmaceutical compositions of the present invention may contain other additives such as a stabilizer, preservative, isotonizing agent, pH-modifier, antioxidant and colorant. Examples of the stabilizer include, but not limited to, sodium bisulfite, glycerin, sodium edetate, sodium citrate and butyl hydroxyanisole. Example of the preservative include, but not limited to, benzalkonium chloride. Example of the isotonizing agent include, but not limited to, sodium chloride, D-mannitol, glucose and glycerin. Example of the pH-modifier include, but not limited to, phosphates such as monosodium phosphate and sodium hydrogenphosphate, sodium hydroxide and hydrochloric acid. Examples of the antioxidant include, but not limited to, vitamin C.

The pharmaceutical compositions of the present invention can be prepared by methods known in the art in any of accelerated-release, controlled-release and delayed-release dosage forms. The concentration of active vitamin D in the compositions of the present invention depends on the dosage form, but generally ranges from about 0.01 ng/ml to about 1 μg/ml, preferably from about 1 to about 100 ng/ml.

In a preferred embodiment, an ophthalmic solution is

prepared by dissolving calcitriol or 22-oxacalcitriol as an active vitamin D in water or ethanol to a final active vitamin D concentration of about 1 to about 100 ng/ml. The pH is typically adjusted to about 5.0-9.0, preferably about 7.0-8.0. When the active vitamin D is less soluble, a solubilizing agent such as Polysorbate 80, propylene glycol, polyvinyl pyrrolidone K30 or Poloxamer 188 may be added. Thus prepared ophthalmic solution is typically applied to the eye, but not limited to, at a dose of one to several drops once to four times per day depending on the condition.

By applying the composition of the present invention to the site at which inflammation is to be prevented, development of an immunoreactive inflammation at that site can be significantly prevented as a result of the inhibition of migration of Langerhans cells that may mediate the development of the inflammation. Even when inflammation has already occurred, it can also be treated. Particularly, an inflammation at the cornea can be treated without lowering the transparency of the cornea.

The following examples further illustrate the present invention, without limiting the same thereto.

EXAMPLES

Example 1

A stock solution of active vitamin D_3 (containing 50 mg/ml of 1α-hydroxyvitamin D_3) was diluted 1000-fold with ethanol, and further diluted 100-fold with an ophthalmic physiological buffer to prepare an ophthalmic composition containing 0.5 μg/ml of 1α-hydroxyvitamin D_3.

Example 2

Calcitriol was diluted 1000-fold with ethanol, and further diluted 100-fold with an ophthalmic oily base consisting of purified sesame oil to prepare an ophthalmic composition containing 0.5 μg/ml of calcitriol.

Example 3

In 10 ml of the ophthalmic composition prepared by the procedures of Example 1 was dissolved 60 mg of vitamin C (L-ascorbic acid-phosphate) to give an ophthalmic composition consisting of a mixed solution containing 6 mg/ml of vitamin C and 0.5 μg/ml of active vitamin D_3.

Test example 1

Calcitriol was dissolved in a base consisting of sodium phosphate, sodium chloride, ethanol and Polysorbate 80 to prepare three solutions at a concentration of 1.0×10^{-6} M, 1.0×10^{-7} M and 1.0×10^{-8} M. Separately, a steroid, dexamethasone sodium was dissolved in the same base to prepare a 0.1% solution.

Each of 30 BALB/c mice got 2 stitches of 10-0 nylon thread in the central region of the cornea of one eye and then was left for migration of Langerhans cells in the corneas thereof. The mice were divided into 6 groups A-F each consisting of 5 animals, in which group A received no instillation, group B received the steroid (St), groups C-E received calcitriol at the three concentrations mentioned above, and group F received the base alone.

The instillation was started at a frequency of three times per day on the day following stitching. Fourteen days

after stitching, the mice were killed and their eyes were extracted to assess the average density of Langerhans cells in the central corneal epithelium by immunostaining for each group. The results are shown in Table 1.

Table 1: Density of Langerhans cells in the central corneal epithelium

Group	Density (cells/mm^2)	p value
A (no instillation)	5.0 ± 0.9	
B (St)	1.0 ± 0.3	< 0.005
C (1.0 x10^{-6} M)	3.3 ± 0.6	N.S.
D (1.0 x10^{-7} M)	2.5 ± 0.7	< 0.05
E (1.0 x10^{-8} M)	2.6 ± 0.5	< 0.025
F (base)	4.6 ± 0.8	

Table 1 shows that the number of Langerhans cells is significantly reduced in groups C-E, which received calcitriol, as compared with group A, which received no instillation, and group F, which received the base alone. In addition, the calcitriol-induced reduction is concentration-independent. This suggests that calcitriol may have an optimal concentration for the inhibition of Langerhans cell migration. Generally, many migration inhibitors for cells involved in immunoreaction have such an optimal concentration.

During this instillation, eyes of the mice were also observed under a slit lamp microscope every second day to

evaluate neovascularization as follows. The length of neovascularization in each of four quadrants of the cornea of each mouse is scored according to the following standard. The scores of neovascularization in four quadrants of each cornea are totalyzed (scores 0-12), and an average of the totalyzed scores for each group is calculated.

Score 0: No neovascularization is observed;

Score 1: Neovascularization does not reach the central region of the cornea (within a 1 mm radius from the center);

Score 2: Neovascularization reaches the central region of the cornea (within a 1 mm radius from the center) but does not reach the center;

Score 3: Neovascularization reaches the central cornea.

The results are shown in Fig. 1, which demonstrates that calcitriol also has an inhibitory effect on neovascularization.

Test example 2

Experiments:

To investigate the mechanism of the inhibitory effect of calcitriol ($1\alpha,25(OH)_2D_3$) on Langerhans cell migration or neovascularization in the corneal epithelium, human corneal epithelial cells were grown in the presence of $1\alpha,25(OH)_2D_3$, after which the number of cells was determined and cytokine production level in the supernatant of the culture medium was also determined.

Human corneal epithelial cells kindly given by

Dr. Sasaki (Toyonaka City Hospital, Osaka) were grown in Dulbecco's modified Eagle's medium (DMEM) /F-12 (1/1) supplemented with 10% fetal bovine serum, 5 μg/ml insulin, 0.1 μg/ml cholera toxin, 10 ng/ml human EGF, 0.5% DMSO and 40 μg/ml gentamicin at a temperature of 37°C and a humidity of 95% under 5% carbon dioxide. The medium was changed twice a week.

When human corneal epithelial cells were confluent, the medium was replaced with serum-free DMEM supplemented with 5 μg/ml insulin, 5 mg/ml transferrin and 5 ng/ml sodium selenite. After a 48 hour incubation, cells were seeded on a 6-well plate at 4×10^6 cells per well and incubated in the presence of 0.1% $1\alpha,25(OH)_2D_3$ in ethanol at concentrations of 1.0×10^{-7} M, 1.0×10^{-11} M and 1.0×10^{-15} M each for two wells. These wells were divided into two groups of 3 wells having different $1\alpha,25(OH)_2D_3$ concentrations, and the number of cells was determined after a 6 hour incubation for one group and a 12 hour incubation for the other group. Then, the culture media were each centrifuged at 150 g for 5 minutes and the supernatant was stored at -80°C. These experiments were performed at least in triplicate.

As a control, the same experiments were performed but in the absence of $1\alpha,25(OH)_2D_3$ (untreated) or in the presence of ethanol without $1\alpha,25(OH)_2D_3$ (solvent).

Cytokine quantitation was performed for interleukin-1α (IL-1α), interleukin-1β (IL-1β) and interleukin-8 (IL-8) using an ELISA kit (R & D System Corp.). The quantitation

limits for the cytokines were 0.5 pg/ml for IL-1α, 1 pg/ml for IL-1β, and 10 pg/ml for IL-8. Colorimetry was performed at 450 nm using EL 308 (Bio-tek Instrument, Wincoski). The results of measurement of the three experiments were assembled and statistically analyzed by unpaired t-test.

Results:

As shown in Fig. 2, no significant difference is found in cell growth between the groups treated with $1\alpha,25(OH)_2D_3$ and the solvent control group. Therefore, the *in vitro* influence of $1\alpha,25(OH)_2D_3$ on the growth of corneal epithelial cells is very weak.

On the other hand, $1\alpha,25(OH)_2D_3$ exhibits an inhibitory effect on production of all of the cytokines IL-1α, IL-1β and IL-8, as shown in Figs. 3 to 5. The effect on IL-1α and IL-1β is especially pronounced. However, the effect on IL-8 is rather poor.

Specifically, Figs. 3 and 4 show that the amounts of IL-1α and IL-1β produced in the absence of $1\alpha,25(OH)_2D_3$ after a 6 hour incubation are 199.8 ± 0.65 pg/10^6 cells and 29.9 ± 2.8 pg/10^6 cells, respectively, and both increase to about 1.5 times after a 12 hour incubation, but the amounts of IL-1α and IL-1β produced after the 6 hour incubation in the presence of $1\alpha,25(OH)_2D_3$ are only 25% and about 77% as compared with the untreated group irrespective of the concentration of $1\alpha,25(OH)_2D_3$. These amounts are shown to scarcely increase even after the 12 hour incubation. Fig. 5 shows that the amount of IL-8 produced in the groups treated with $1\alpha,25(OH)_2D_3$ significantly decrease

concentration-dependently after a 6 hour incubation as compared with the untreated group, but does not significantly decrease after a 12 hour incubation as compared with the untreated group.

These results show that $1\alpha,25(OH)_2D_3$ has a very strong *in vitro* inhibitory effect on IL-1 production in human corneal epithelial cells, but its inhibitory effect on IL-8 production is relatively weak. This is the first finding demonstrating that $1\alpha,25(OH)_2D_3$ directly inhibits IL-1 production in corneal epithelial cells. These results indicate that the inhibition of Langerhans cell migration in the corneal epithelium by $1\alpha,25(OH)_2D_3$ results from the inhibition of IL-1 production in the corneal epithelium by $1\alpha,25(OH)_2D_3$, because strong *in vitro* inhibitory effects on IL-1 production correspond to strong *in vivo* inhibitory effects on Langerhans cell migration and relatively weak *in vitro* inhibitory effects on IL-8 production correspond to relatively weak *in vivo* inhibitory effects on neovascularization.

CLAIMS

1. A Langerhans cell migration inhibitor comprising active vitamin D as an active ingredient.

2. The Langerhans cell migration inhibitor of Claim 1 wherein the active vitamin D is vitamin D_3 or a derivative or analog of vitamin D_3.

3. The Langerhans cell migration inhibitor of Claim 2 wherein the active vitamin D is vitamin D_3.

4. The Langerhans cell migration inhibitor of Claim 3 wherein the active vitamin D_3 is calcitriol or 22-oxacalcitriol.

5. The Langerhans cell migration inhibitor of any one of Claims 1 to 4 wherein the inhibitor is in the form of an ophthalmic solution.

6. The Langerhans cell migration inhibitor of Claim 5 wherein the inhibitor is used for the prevention and/or treatment of ocular inflammation.

7. The Langerhans cell migration inhibitor of Claim 6 wherein the ocular inflammation is keratoconjunctivitis.

8. The Langerhans cell migration inhibitor of Claim 5 wherein the inhibitor is used for the prevention of ocular inflammation.

9. The Langerhans cell migration inhibitor of Claim 8 wherein the ocular inflammation is phlyctenular keratitis or corneal infiltration.

10. The Langerhans cell migration inhibitor of Claim 5 wherein the inhibitor prevents and/or treats an

ocular inflammation by inhibiting the production of interleukin-1 in corneal epithelial cells.

ABSTRACT

The present invention provides a Langerhans cell migration inhibitor comprising an active vitamin D as an active ingredient. The Langerhans cell migration inhibitor is useful for preventing immunoreactive inflammation of the skin or cornea and treating it after it occurs, and has less side effects.

Fig. 1

Fig. 2

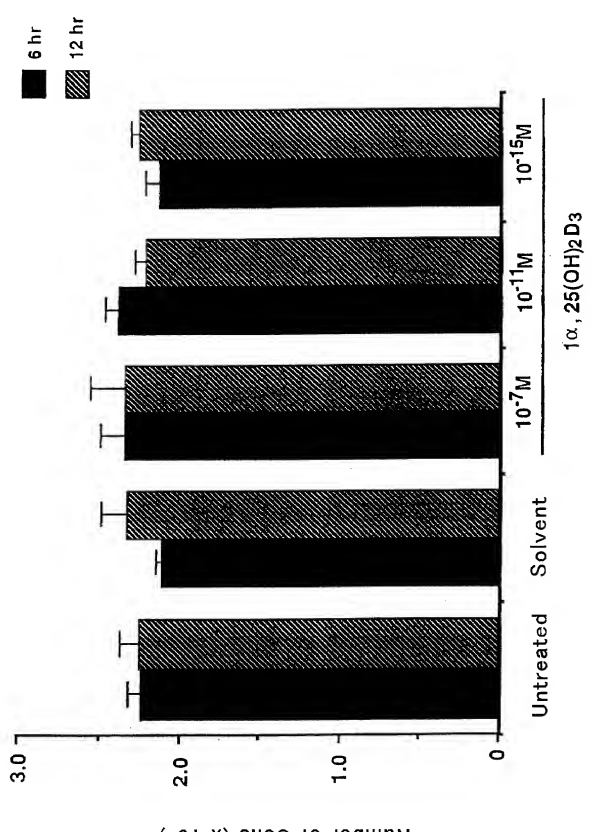


Fig. 3

Fig. 4

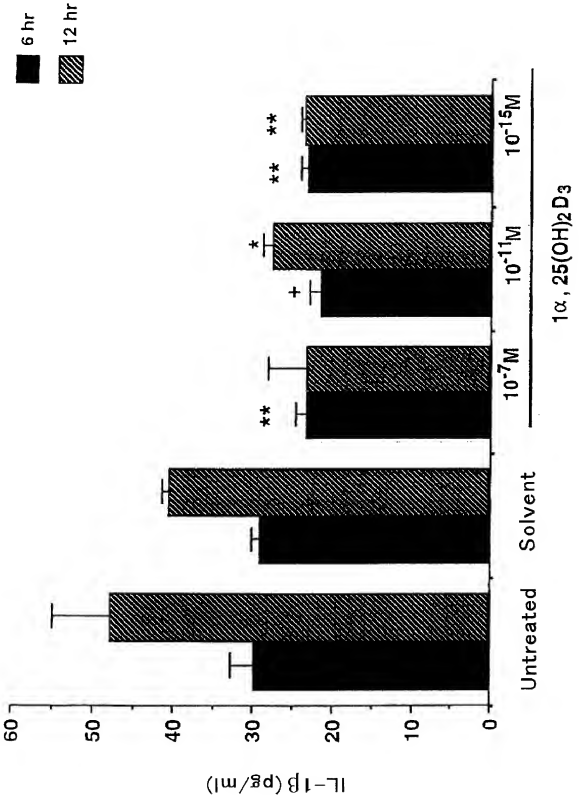
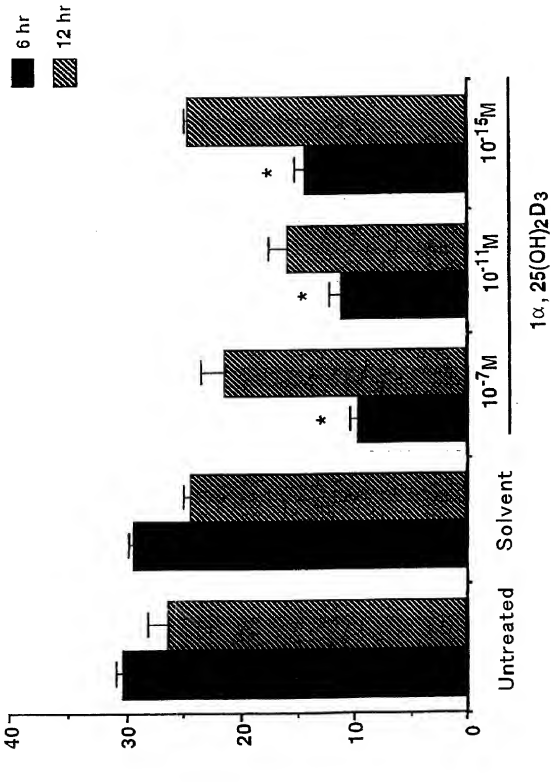

6 hr
12 hr
IL-1β (pg/ml)
60
50
40
30
20
10
0
Untreated
Solvent
10-7M
10-11M
10-15M
1α, 25(OH)2 D3
**
+
*
**
**

Fig. 5


6 hr
12 hr
IL-8 (pg/ml)
40
30
20
10
0
Untreated
Solvent
10^{-7}M
10^{-11}M
10^{-15}M
1α, 25(OH)$_2$D$_3$
*
*
*

Page 1 of 2 Pages [x] Original [ ] Supplemental Atty. Docket:

Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

LANGERHANS CELL MIGRATION INHIBITORS

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on _____, as U.S. Appln. No. _____*; or
[x] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP99/00837 filed Feb. 24, 1999; entry requested on _____*; national stage application received U.S. Appln. No. _____*; §371/§102(e) date _____*
(* if known)

and was amended on _____ (if applicable).
(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 and 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	YES	NO
44757/1998	Japan	26/2/1998	[x]	[ ]
			[ ]	[ ]

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or prior PCT application(s) designating the U.S. listed below, or under §119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practioners associated with Customer Number 001444

Direct all correspondence to the address associated with **Customer Number 001444**, i.e.,

BROWDY AND NEIMARK, P.L.L.C.
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The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from _____ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Page 2 of 2 Pages

Atty. Docket:

Title: LANGERHANS CELL MIGRATION INHIBITORS

U.S. Application filed _____, Serial No. _____

PCT Application filed February 24, 1999 Serial No. PCT/JP99/00833

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-60

FULL NAME OF FIRST INVENTOR	INVENTOR'S SIGNATURE	DATE
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FULL NAME OF THIRD JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.